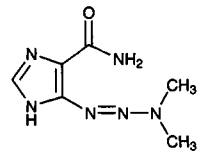
Dacarbazine



Molecular formula: $C_6H_{10}N_6O$
Molecular weight: 182.19
CAS Registry No.: 4342-03-4
Merck Index: 2866
Lednicer No.: 2 254

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 5 mL MeOH:chloroform 3:1, vortex for 60 s, let stand at 4° for 15 min, centrifuge at 4° at 1300 g for 10 min, inject an aliquot of the supernatant. (Caution ! Chloroform is a carcinogen !)

HPLC VARIABLES

Column: 300 × 3.9 10µm. µBondapak phenyl
Mobile phase: Gradient. MeOH:0.1% pH 5.5 ammonium formate buffer:water 5:90:5, for 0.5 min, to 30:40:30 over 1 min, maintain at 30:40:30 for 7.5 min, re-equilibrate at initial conditions for 4 min.
Flow rate: 2
Injection volume: 20–100
Detector: UV 254

CHROMATOGRAM

Retention time: 6.8
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: 5-aminoimidazole-4-carboxamide, 2-azahypoxanthine

KEY WORDS

plasma

REFERENCE

Tate,P.S.; Briele,H.A. Reversed-phase high-performance liquid chromatography of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide and metabolites, *J.Chromatogr.*, **1986**, *374*, 421–424.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 325

CHROMATOGRAM

Retention time: 3.16
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 0.5-1 mL Plasma + 100 μL 100 μg/mL 3-methylxanthine, filter (Amicon CF-25), inject a 60 μL aliquot of the ultrafiltrate. Urine. 1 mL Urine + 100 μL 100 μg/mL 3-methylxanthine, dilute six-fold with water, filter (25 mm Swinnex), inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 45 × 4 10 μm Spherisorb octadecylsilyl
Column: 300 × 4 μBondapak C18

Mobile phase: Gradient. A was 500 mM sodium acetate adjusted to pH 7.0 with 10% phosphoric acid. B was MeCN:50 mM sodium acetate adjusted to pH 5.5 with concentrated phosphoric acid 25:75. A:B 100:0 for 5 min, then to 5:95 over 3 min, maintain at 5:95 for 6 min. Re-equilibrate at initial conditions for 6 min.
Injection volume: 50-60
Detector: UV 280

CHROMATOGRAM
Retention time: 12.3
Internal standard: 3-methylxanthine (11.6)
Limit of detection: 5000 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 2-azahypoxanthine, 5-aminoimidazole-4-carboxamide

KEY WORDS
plasma; protect from light; ultrafiltrate

REFERENCE
Fiore,D.; Jackson,A.J.; Didolkar,M.S.; Dandu,V.R. Simultaneous determination of dacarbazine, its photolytic degradation product, 2-azahypoxanthine, and the metabolite 5-aminoimidazole-4-carboxamide in plasma and urine by high-pressure liquid chromatography, *Antimicrob.Agents Chemother.*, **1985**, *27*, 977–979.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 323.5

CHROMATOGRAM
Retention time: 3.6

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 220 × 4.6 5 μm silica (Brownlee)
Mobile phase: MeCN:6.25 mM NaH_2PO_4 adjusted to pH 3.0 with concentrated phosphoric acid 40:60
Flow rate: 1
Injection volume: 50
Detector: UV 216

CHROMATOGRAM
Retention time: 5.7
Limit of detection: 12.5 ng/mL

OTHER SUBSTANCES
Simultaneous: doxorubicin, ondansetron
Noninterfering: degradation products

KEY WORDS
injections; 5% dextrose

REFERENCE
King,D.T.; Stewart,J.T. HPLC determination of dacarbazine, doxorubicin, and ondansetron mixture in 5% dextrose injection on underivatized silica with an aqueous-organic mobile phase, *J.Liq.Chromatogr.*, **1993**, *16*, 2309–2323.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 2.32

OTHER SUBSTANCES
Simultaneous: cimetidine (UV 228), cisplatin (UV 198), granisetron

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: reaction mixtures
Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 4.6 5 μm Microsorb C8
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeOH:0.4 g/L $(NH_4)H_2PO_4$ + 0.1% triethylamine (pH 10.0) 30:70
Flow rate: 1
Injection volume: 20
Detector: UV 325

CHROMATOGRAM

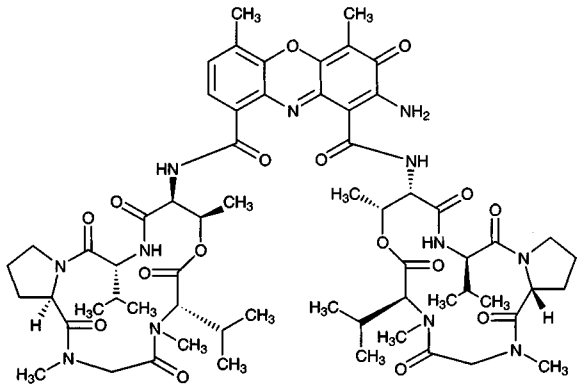
Retention time: 6.4
Limit of detection: 500 ng/mL

REFERENCE

Lunn,G.; Rhodes,S.W.; Sansone,E.B.; Schmuff,N.R. Photolytic destruction and polymeric resin decontamination of aqueous solutions of pharmaceuticals, *J.Pharm.Sci.*, **1994**, *83*, 1289–1293.

Dactinomycin

Molecular formula: $C_{62}H_{86}N_{12}O_{16}$
Molecular weight: 1255.44
CAS Registry No.: 50-76-0
Merck Index: 2867



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 2 M HCl, vortex for 10 s, add 3 mL ethyl acetate saturated with water, vortex for 30 s, centrifuge at 300 g for 2 min. Remove 2 mL of the supernatant, extract the aqueous phase again with 3 mL ethyl acetate saturated with water. Combine the organic layers and evaporate them to dryness under a stream of air, reconstitute with 1 mL MeOH, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm Bondapak C18
Mobile phase: MeCN:30 mM pH 4.6 sodium acetate 35:65
Flow rate: 1.5
Injection volume: 100
Detector: UV 436

CHROMATOGRAM

Retention time: 6.4
Limit of detection: 40 ng/mL

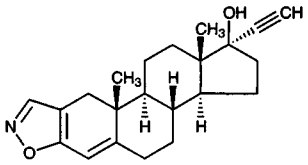
KEY WORDS

plasma; use siliconized glass; do not use plastic

REFERENCE

Schneebaum,S.; Bies,J.M.; Briele,H.A. Reversed-phase high-performance liquid chromatography of dactinomycin, *J.Chromatogr.*, **1988**, *427*, 166–171.

Danazol



Molecular formula: $C_{22}H_{27}NO_2$
Molecular weight: 337.46
CAS Registry No.: 17230-88-5
Merck Index: 2875
Lednicer No.: 2 157

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 100 µL 10 µg/mL IS + 5 mL pentane:dichloromethane 80:20, shake for 10 min on a reciprocal shaker at 150 rpm, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 µL mobile phase. Inject an aliquot onto column A and elute to waste with mobile phase, after 1.4 min direct the effluent from column A onto column B, after another 2.6 min remove column A from the circuit. Elute column B with mobile phase and monitor the effluent. Continue to elute column A with mobile phase to remove late-eluting peaks.

HPLC VARIABLES

Column: A 150 × 3.9 5 µm Spherisorb C8; B 150 × 4.6 5 µm Spherisorb ODS-2
Mobile phase: MeCN:water 55:45
Flow rate: 1.6
Detector: UV 285

CHROMATOGRAM

Retention time: 9.5
Internal standard: 1,4-androstadiene-3,17-dione (4.2)
Limit of detection: 1 ng/mL

KEY WORDS

serum; column-switching; heart-cut; pharmacokinetics

REFERENCE

Selinger,K.; Hill,H.M.; Anslow,J.A.; Gash,D. A liquid chromatographic method for the determination of danazol in human serum, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 79–84.

SAMPLE

Matrix: bulk
Sample preparation: Prepare a 0.1% solution in mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 LiChrosorb 10-RP-18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 20
Detector: UV 240, 254, 284

CHROMATOGRAM

Retention time: 15.9
Limit of detection: 0.05% of danazol

OTHER SUBSTANCES

Simultaneous: impurities, ethisterone, isodanazol

REFERENCE

Balogh,G.; Csizér,.; Ferenczy,G.G.; Halmos,Z.; Herényi,B.; Horváth,P.; Laukó,A.; Görög,S. Estimation of impurity profiles of drugs and related materials. 12. Isolation and identification of an isomeric impurity in danazol, *Pharm.Res.*, **1995**, *12*, 295–298.

SAMPLE

Matrix: formulations

Sample preparation: Crush tablets, weigh out amount equivalent to 10 mg steroid, dissolve in 10 mL MeOH, sonicate for 15 min, filter. 1 mL Filtrate + 5 mL MeOH + 4 mL water, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS

Mobile phase: Gradient. MeOH:water from 70:30 to 100:0 over 15 min, maintain at 100:0 for 15 min.

Flow rate: 1

Injection volume: 25

Detector: UV 280

CHROMATOGRAM

Retention time: 14.0

OTHER SUBSTANCES

Simultaneous: (at UV 240 nm) boldenone, boldenone acetate, boldenone undecylenate, clostebol acetate, fluoxymesterone, methandriol, methandriol-3-acetate, methandriol dipropionate, methandrostenolone, methyltestosterone, nandrolone, nandrolone decanoate, nandrolone phenylpropionate, nandrolone propionate, stanolone, stanozolol, testosterone, testosterone acetate, testosterone cypionate, testosterone enanthate, testosterone isobutyrate, testosterone propionate, testosterone undecanoate

Noninterfering: oxandrolone, oxymetholone, testosterone decanoate, testosterone isocaproate

KEY WORDS

tablets

REFERENCE

Lurie,I.S.; Sperling,A.R.; Meyers,R.P. The determination of anabolic steroids by MECC, gradient HPLC, and capillary GC, *J.Forensic Sci.*, **1994**, *39*, 74–85.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb S5-ODS2 (A), 125 × 4 5 μm LiChrospher ODS-3 (B), 250 × 4.6 Partisil 10 ODS-3 (C)

Mobile phase: MeCN:water 65:35 (A), MeCN:MeOH:water 40:30:30 (B and C)

Flow rate: 1 (A), 1.5 (B), 2 (C)

Injection volume: 20 (A), 100 (B and C)

Detector: UV 270 (A), UV 280 (C)

CHROMATOGRAM

Internal standard: testosterone propionate

REFERENCE

Galia,E.; Nicolaides,E.; Hörter,D.; Löbenberg,R.; Reppas,C.; Dressman,J.B. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs, *Pharm.Res.*, **1998**, *15*, 698–705.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in MeOH.

HPLC VARIABLES
Guard column: 70 × 2.1 Whatman CO:Pell ODS
Column: 300 × 3.9 Bondex C18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 5
Detector: UV 280

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: methyltestosterone, nandrolone, methandrostenolone, boldenone, testosterone, fluoxymesterone

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and spectral analysis of the 17-hydroxy anabolic steroids, *J.Chromatogr.Sci.*, **1990**, *28*, 162–166.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 26.6

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine
Interfering: biphenyl

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 10 μm Partisil 10 ODS
Mobile phase: MeCN:MeOH:water 40:30:30
Flow rate: 1
Detector: UV 280

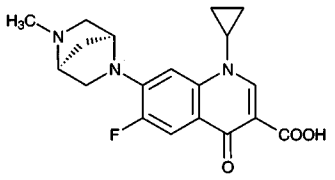
CHROMATOGRAM

Retention time: 8

REFERENCE

Mithani,S.D.; Bakatselou,V.; TenHoor,C.N.; Dressman,J.B. Estimation of the increase in solubility of drugs as a function of bile salt concentration, *Pharm.Res.*, **1996**, *13*, 163–167.

Danofloxacin



Molecular formula: $C_{19}H_{20}FN_3O_3$, $C_{19}H_{20}FN_3O_3.CH_4O_3S$ (monomethane sulfonate)

Molecular weight: 357.38

CAS Registry No.: 112398-08-0, 119478-55-6 (monomethane sulfonate)

Merck Index: 2876

SAMPLE

Matrix: blood, gastric contents; tissue

Sample preparation: Blood. Mix 1 mL plasma with IS and MeCN, centrifuge at 2800 g for 20 min, inject an aliquot. Tissue. Centrifuge 500 mg homogenized lung tissue at 1820 g, separate the sediment, reconstitute it with 500 μL water, vortex for 20 s. Mix with 5 μg/g IS in MeCN (sample:MeCN 40:60). Vortex for 20 s, centrifuge at 1820 g. Collect the supernatant and dilute with water (sample:water 30:70). Centrifuge the mixture and inject an aliquot of the supernatant. Mesenteric lymph node, brain, interdigital skin. Centrifuge 500 mg homogenized tissue at 1820 g, mix sediment with IS, vortex, mix with 1 mL 2 M pH 8.5 K_2HPO_4, vortex, homogenize (Ultraturrax) for 20 s. Add 5 mL ethyl acetate:isopropanol 70:30, mix for 10 min. Centrifuge at 1820 g, remove 3 mL portion, dry at 50° under a stream of nitrogen, reconstitute the residue in mobile phase, inject an aliquot. Intestinal contents. Condition a Sep-Pak C18 SPE cartridge with 3 mL MeOH and 3 mL 25 mM pH 3.0 K_2HPO_4. Centrifuge 1 g gastrointestinal contents at 1820 g, add IS, vortex, mix with 2 M pH 8.5 K_2HPO_4. Add 10 mL ethyl acetate:isopropanol 70:30, mix for 10 min, centrifuge at 1820 g, remove the supernatant, dry at 50° under nitrogen. Reconstitute the residue with 1 mL 25 mM pH 3.0 K_2HPO_4, sonicate for 2 min, vortex for 20 s. Add to the SPE cartridge, wash with 3 mL 35 mM pH 3.0 K_2HPO_4, dry under vacuum, elute with 3 mL MeOH. Dry the eluate under a stream of nitrogen at 50°, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 5 μm Techsphere C18

Mobile phase: MeCN:10 mM pH 3.0 phosphate buffer

Flow rate: 1

Detector: F ex 280 em 440

CHROMATOGRAM

Internal standard: CP.71.755 (a structural analogue of danofloxacin)

Limit of detection: 10 ng/mL (plasma), 40 ng/g (tissue, all gastrointestinal fluids)

KEY WORDS

SPE; plasma; mesenteric lymph nodes; duodenum; jejunum; ileum; colon; brain; interdigital skin; sheep; pharmacokinetics

REFERENCE

McKellar,Q.A.; Gibson,I.F.; McCormack,R.Z. Pharmacokinetics and tissue disposition of danofloxacin in sheep, *Biopharm.Drug Dispos.*, **1998**, *19*, 123–129.

SAMPLE

Matrix: tissue

Sample preparation: 500 mg Sample + 5 mL extraction solvent, mix at high speed for 10 s, homogenize at high speed (Polytron homogenizer) for 30 s. Re-suspend the tissue by mixing at high speed for 10 s, incubate in a 50 ± 5° water bath for 90 ± 10 min. Centrifuge at 1200 g for 10 min, inject a 20 μL aliquot of the supernatant. (Extraction solvent was 15 mM $HClO_4$ and 15 mM phosphoric acid in MeOH:water 50:50.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Inertsil C8 (GL Sciences, Japan)
Mobile phase: MeCN:50 mM pH 3.5 phosphate buffer 12:88 (Buffer was prepared by dissolving 6.62 g monosodium phosphate in 900 mL water, pH was adjusted to 3.5 with phosphoric acid and solution was diluted to 1 L with water.)
Column temperature: 35 ± 0.5
Flow rate: 1
Injection volume: 20
Detector: F ex 280 em 440

CHROMATOGRAM

Retention time: 18.5
Limit of quantitation: 10 ng/g

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: ciprofloxacin, enrofloxacin, norfloxacin, ofloxacin

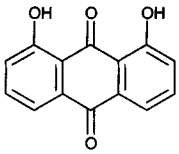
KEY WORDS

liver; muscle; kidney; fat; cow; chicken

REFERENCE

Strelevitz,T.J.; Linhares,M.C. Simultaneous determination of danofloxacin and N-desmethyldanofloxacin in cattle and chicken edible tissues by liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1996**, *675*, 243–250.

Danthron



Molecular formula: $C_{14}H_8O_4$
Molecular weight: 240.22
CAS Registry No.: 117-10-2
Merck Index: 2878

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

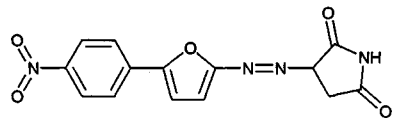
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, py-

rilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Dantrolene



Molecular formula: $C_{14}H_{10}N_4O_5$
Molecular weight: 314.26
CAS Registry No.: 7261-97-4, 24868-20-0 (sodium salt hemiheptahydrate)
Merck Index: 2879
Lednicer No.: 2 242

SAMPLE

Matrix: blood
Sample preparation: 50 μL Plasma + 100 μL 10 μg/mL methyldantrolene in MeCN, vortex for 30 s, centrifuge at 1500 g for 5 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 10 μm LiChrosorb RP-18
Mobile phase: MeCN:20 mM glycine 35:45, final pH adjusted to 3.6 with phosphoric acid
Flow rate: 2
Injection volume: 25
Detector: UV 375

CHROMATOGRAM

Retention time: 6.4
Internal standard: methyldantrolene (11.2) (synthesis details in paper)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: tetracycline, benzodiazepines

KEY WORDS

plasma; human; rat; pharmacokinetics

REFERENCE

Lalande,M.; Mills,P.; Peterson,R.G. Determination of dantrolene and its reduced and oxidized metabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *430*, 187–191.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Blood + 1 mL MeCN, centrifuge. Remove 850 μL of the supernatant and evaporate under a stream of nitrogen, reconstitute in 25 μL 100 mM NaOH and 75 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 5 μm LiChrosorb RP8 RT, 125-4
Mobile phase: MeCN:pH 6.8 phosphate buffer 45:55
Flow rate: 1
Detector: UV 405

OTHER SUBSTANCES

Noninterfering: sodium taurocholate

KEY WORDS

rat; pharmacokinetics

REFERENCE

Poelma,F.G.J.; Tukker,J.J.; Crommelin,D.J.A. Intestinal absorption of drugs. I: The influence of taurocholate on the absorption of dantrolene in the small intestine of the rat, *J.Pharm.Sci.*, **1989**, *78*, 285–289.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.04 (A), 5.23 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

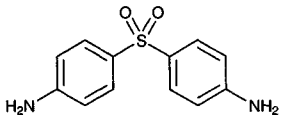
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Dapsone



Molecular formula: $C_{12}H_{12}N_2O_2S$
Molecular weight: 248.31
CAS Registry No.: 80-08-0
Merck Index: 2885
Lednicer No.: 1 139

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL Plasma with 5 μL 50 μg/mL IS in MeOH, 200 mg NaCl, 200 μL 1.5 M NaOH, and 7 mL diethyl ether. Shake for 30 min in a mechanical shaker at 220 ±10 cycles/min, centrifuge at 1000 g for 10 min, remove a 5 mL aliquot of the organic layer, evaporate under a stream of air, add 50 μL mobile phase and 50 μL n-hexane to the residue, vortex for 10 s, inject a 20 μL aliquot of the lower phase.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher 100 RP-8
Mobile phase: MeOH:water 30:70
Flow rate: 1
Injection volume: 20
Detector: UV 286

CHROMATOGRAM

Retention time: 3.82
Internal standard: phenacetin (9.1)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Extracted: monoacetyldapsone
Simultaneous: acetaminophen, aspirin, clofazimine, diazepam, thalidomide, trimethoprim, sulfamethoxazole
Noninterfering: quinine, rifampin

KEY WORDS

plasma

REFERENCE

Queiroz,R.H.; Dreossi,S.A.; Carvalho,D. A rapid, specific, and sensitive method for the determination of acetylation phenotype using dapsone, *J.Anal.Toxicol.*, **1997**, *21*, 203–207.

SAMPLE

Matrix: blood
Sample preparation: Mix 50 μL plasma, filter paper-absorbed plasma, or whole blood with 1 μg IS. Dry the filter paper-absorbed samples under air for 30 min, cut blots into small pieces. Add 200 μL ammonia to liquid or dry sample, vortex for 5 s, extract with 5 mL ethyl acetate:MTBE 50:50, centrifuge, separate, evaporate the organic phase to dryness at 37°. Reconstitute the residue with 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm CN RP-18 (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:glacial acetic acid 17.5:81:5 containing 2 g/L l-octanesulfonic acid
Injection volume: 50

Detector: UV 274

CHROMATOGRAM
Retention time: 6
Internal standard: acetanilide (4.5)
Limit of detection: 15 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; whole blood

REFERENCE
Mberu,E.K.; Muhia,D.K.; Minyiri,G.O.; Njonge,E.W.; Watkins,W.M. Measurement of physiological concentrations of dapsone and its monoacetyl metabolite: a miniaturised assay for liquid or filter paper-absorbed samples, *J.Chromatogr.B*, **1996**, *677*, 385–387.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 20 μL MeOH:60% perchloric acid 50:50, vortex for 1 min, centrifuge at 9950 g for 2 min, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Magnusphere C18 (Magnus Scientific)
Mobile phase: MeOH:67 mM pH 5.9 phosphate buffer 47:23
Flow rate: 1.2
Injection volume: 20
Detector: UV 295

CHROMATOGRAM
Retention time: 3.3
Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Philip,P.A.; Roberts,M.S.; Rogers,H.J. A rapid method for determination of acetylation phenotype using dapsone, *Br.J.Clin.Pharmacol.*, **1984**, *17*, 465–469.

SAMPLE
Matrix: blood
Sample preparation: 150 μL Whole blood or plasma + 25 μL monopropionyldapsone in EtOH, mix at 2200 vibrations/min for 10 min, add 100 μL 2 M NaOH, add 3 mL MTBE, shake mechanically for 15 min, centrifuge at 1200 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, vortex for 20 s, centrifuge at 1200 g for 2 min, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ
Mobile phase: MeCN:MeOH:25 mM pH 2.3 phosphate buffer 20:10:70
Flow rate: 1.2

Injection volume: 80
Detector: UV 286

CHROMATOGRAM
Retention time: 3.9
Internal standard: monopropionyldapsone (7.5) (Reflux dapsone with propionic anhydride in ethyl acetate for 10 min, purify by preparative TLC.)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: monoacetyldapsone, pyrimethamine
Noninterfering: chloroquine, quinine, sulfamethoxazole, trimethoprim, acetaminophen
Interfering: proguanil

KEY WORDS
whole blood; plasma

REFERENCE
Lemnge,M.M.; Ronn,A.; Flachs,H.; Bygbjerg,I.C. Simultaneous determination of dapsone, monoacetyldapsone and pyrimethamine in whole blood and plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *613*, 340–346.

SAMPLE
Matrix: blood
Sample preparation: Whole blood. 150 μL Whole blood + 25 μL 800 ng/mL monopropionyldapsone + 1.2 mL 200 mM NaOH + 5 mL MTBE, mix for 25 min, centrifuge at 1600 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot. Dried blood. Let 150 μL blood dry on filter paper. Cut paper into small pieces, add 25 μL 800 ng/mL monopropionyldapsone, add 1.2 mL 200 mM NaOH, mix gently for 30 min, add 5 mL MTBE, mix for 25 min, centrifuge at 1600 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-ABZ (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ
Mobile phase: MeCN:MeOH:buffer 14:7:49 (Buffer was 25 mM phosphate adjusted to pH 2.3 with orthophosphoric acid.)
Flow rate: 1.2
Injection volume: 80
Detector: UV 286

CHROMATOGRAM
Retention time: 3.6
Internal standard: monopropionyldapsone (7.1)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: monoacetyldapsone, metabolites, pyrimethamine
Noninterfering: acetaminophen, chloroquine, quinine, sulfadoxine, sulfamethoxazole, trimethoprim

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KEY WORDS
whole blood; dried blood

REFERENCE

Ronn,A.M.; Lemnge,M.M.; Angelo,H.R.; Bygbjerg,I.C. High-performance liquid chromatography determination of dapsone, monoacetyldapsone, and pyrimethamine in filter paper blood spots, *Ther.Drug Monit.*, **1995**, *17*, 79–83.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 4 μg thiopental (in MeOH) + 2 mL 34 mg/mL pH 5.5 KH_2PO_4 + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under reduced pressure at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 10000 g for 5 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPak C18
Mobile phase: MeOH:THF:0.68 mg/mL pH 2.6 KH_2PO_4 65:5:30
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 293

CHROMATOGRAM

Retention time: 2.9
Internal standard: thiopental (6.0)

KEY WORDS

plasma

REFERENCE

Tracqui,A.; Gutbub,A.M.; Kintz,P.; Mangin,P. A case of acute dapsone poisoning: Toxicological data and review of the literature, *J.Anal.Toxicol.*, **1995**, *19*, 229–235.

SAMPLE

Matrix: blood, saliva

Sample preparation: Serum. 200 μL Serum + 500 μL mobile phase, shake for 10 min, centrifuge at 2400 g for 5 min, inject 20 μL of the organic layer. Saliva. 200 μL Saliva + 200 μL 1.4 M pH 7.5 phosphate buffer + 1 mL mobile phase, shake for 10 min, centrifuge at 2400 g for 5 min, inject a 20 μL aliquot of the organic layer.

HPLC VARIABLES

Column: 100 × 3 5 μm Chromspher Si (Chrompack)
Mobile phase: 1,2-Dichloroethane:n-butyl acetate (50% water saturated) 10:90
Flow rate: 1
Injection volume: 20
Detector: F ex 290 em 380

CHROMATOGRAM

Retention time: 0.92
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: monoacetyldapsone

KEY WORDS

serum; normal phase; pharmacokinetics

REFERENCE
Pieters,F.A.J.M.; Vincken,B.J.; Zuidema,J. Dapsone and monoacetyldapsone determined in serum and saliva by a sensitive high-performance liquid chromatographic method with a single extraction step, *J.Chromatogr.*, **1987**, *422*, 322–327.

SAMPLE
Matrix: blood, saliva
Sample preparation: Serum. 200 μL Serum + 100 μL 100 μg/mL diazoxide in buffer + 50 μL 20% perchloric acid, vortex, centrifuge at 2000 g for 5 min, inject a 250 μL aliquot of the supernatant onto column A, then inject 700 μL buffer onto column A, elute the contents of column A onto column B with mobile phase for 1 min, remove column A from circuit, elute column B with mobile phase, monitor the effluent from column B. Backflush column A with 2 mL MeOH:buffer 50:50 then forward flush with 1 mL buffer. Saliva. Centrifuge saliva at 3000 g for 4 min. 100 μL Supernatant + 150 μL water + 100 μL 5 μg/mL diazoxide in buffer, vortex, inject a 250 μL aliquot onto column A, then inject 700 μL buffer onto column A, elute the contents of column A onto column B with mobile phase for 1 min, remove column A from circuit, elute column B with mobile phase, monitor the effluent from column B. Backflush column A with 2 mL MeOH:buffer 50:50 then forward flush with 1 mL buffer. (Buffer was 50 mM pH 4.6 $(NH_4)H_2PO_4$.)

HPLC VARIABLES
Column: A 30 mm long 30-40 μm C18; B 10 × 4.6 5 μm Spherisorb S5 ODS-1 C18 end-capped + 250 × 4.6 5 μm Spherisorb S5 ODS-1 C18 end-capped
Mobile phase: MeCN:buffer 12:88 (Buffer was 50 mM pH 4.6 $(NH_4)H_2PO_4$.)
Column temperature: 40
Flow rate: 2
Injection volume: 250
Detector: UV 295

CHROMATOGRAM
Retention time: 6.8
Internal standard: diazoxide (8.5)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; column-switching

REFERENCE
Moncrieff,J. Determination of dapsone in serum and saliva using reversed-phase high-performance liquid chromatography with ultraviolet or electrochemical detection, *J.Chromatogr.B*, **1994**, *654*, 103–110.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or 100-500 μL urine + 50 μL 20 μg/mL m-aminophenyl sulfone in MeOH + 100 μL 1 M NaOH + 350 μL water + 3 mL dichloromethane, vortex for 1 min, centrifuge at 950 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 30-50 μL mobile phase, inject a 10-15 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Hibar LiChrosorb RP-18
Mobile phase: MeCN:water:acetic acid 25:73:2
Column temperature: 40

Flow rate: 1.3
Injection volume: 10-15
Detector: UV 250

CHROMATOGRAM
Retention time: 4.5
Internal standard: m-aminophenyl sulfone (6.2)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: monoacetyldapsone

KEY WORDS
plasma

REFERENCE
Horai,Y.; Ishizaki,T. Rapid and sensitive liquid chromatographic method for the determination of dapsone and monoacetyldapsone in plasma and urine, *J.Chromatogr.*, **1985**, *345*, 447–452.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 12.583

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: eggs, milk, tissue

Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)

Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5

Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 11.0

Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: sulfacetamide, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline, sulfathiazole, sulfatroxazole

Noninterfering: chloramphenicol, trimethoprim

KEY WORDS

post-column reaction; meat; column-switching; dialysis

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:MeOH:1 M perchloric acid:water 30:9:0.8:95
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.81

OTHER SUBSTANCES
Simultaneous: amodiaquine, chloroquine, primaquine, pyrimethamine, quinidine, quinine, sulfadoxine, sulfalene, sulfamethoxazole

REFERENCE
Dua,V.K.; Sarin,R.; Sharma,V.P. Sulphadoxine concentrations in plasma, red blood cells and whole blood in healthy and *Plasmodium falciparum* malaria cases after treatment with Fansidar using high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1317–1323.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

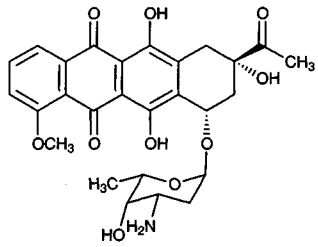
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, na-

lorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Daunorubicin



Molecular formula: $C_{27}H_{29}NO_{10}$
Molecular weight: 527.53
CAS Registry No.: 20830-81-3, 23541-50-6 (HCl)
Merck Index: 2890

SAMPLE

Matrix: blood
Sample preparation: Plasma + 1 mL pH 8.4 phosphate buffer + 8 mL chloroform:1-heptanol 90:10, shake mechanically for 30 min, centrifuge at 3300 rpm for 10 min. Remove the lower organic layer and evaporate it to 2 mL under a stream of nitrogen. Add the residue to 200 µL 300 mM phosphoric acid, vortex. Remove the aqueous phase and add it to 2 mL n-hexane, vortex, centrifuge, inject a 100-150 µL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4 7 µm Lichrosorb RP-18
Mobile phase: MeOH:water 70:30 containing 0.5% acetic acid and 2.5 mM sodium heptanesulfonate
Flow rate: 1.2
Injection volume: 100-150
Detector: UV 254

CHROMATOGRAM

Internal standard: daunorubicin

OTHER SUBSTANCES

Extracted: epirubicin

KEY WORDS

plasma; daunorubicin is IS

REFERENCE

Hu,O.Y.-P.; Chang,S.-P.; Jame,J.-M.; Chen,K.-Y. Pharmacokinetic and pharmacodynamic studies with 4'-epi-doxorubicin in nasopharyngeal carcinoma patients, *Cancer Chemother.Pharmacol.*, **1989**, *24*, 332–337.

SAMPLE

Matrix: blood
Sample preparation: Condition a C2 SPE cartridge with 1 mL MeOH, 500 µL water and 500 µL buffer. 1 mL Plasma + 500 µL water, mix, add to SPE cartridge, wash with 500 µL buffer, elute contents of SPE cartridge with mobile phase onto column for 1 min, remove SPE cartridge, elute column with mobile phase, monitor the effluent. (Buffer was 19 mM NaH_2PO_4 adjusted to pH 4.0 with 100 mM phosphoric acid:MeCN 90:10.)

HPLC VARIABLES

Guard column: 50 × 5 10 µm LiChrosorb RP-18
Column: 100 × 5 5 µm Apex II ODS (Jones Chromatography)
Mobile phase: MeCN:buffer 1:2.25 (Buffer was 19 mM NaH_2PO_4 adjusted to pH 4.0 with 100 mM phosphoric acid.)
Flow rate: 1
Injection volume: 1000
Detector: F ex 480 em 580

CHROMATOGRAM
Retention time: 22
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: epirubicin

KEY WORDS
plasma; SPE; daunorubicin is IS

REFERENCE
Dobbs,N.A.; Twelves,C.J. Measurement of epidoxorubicin and its metabolites by high-performance liquid chromatography using an advanced automated sample processor, *J.Chromatogr.*, **1991**, *572*, 211–217.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma or blood + 3 mL 100 mM pH 9.5 ammonia-ammonium chloride buffer + 13.5 mL chloroform:MeOH 2:1, shake mechanically for 30 min, centrifuge at 3000 g for 10 min, repeat the extraction with 9 mL chloroform. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30°, reconstitute the residue in 3 mL chloroform:MeOH 2:1, evaporate this mixture, reconstitute the residue in 300 μL mobile phase, centrifuge a 75 μL aliquot at 10000 g for 1 min, inject the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm STR ODS-M (Shimadzu)
Mobile phase: MeCN:buffer 30:70 (Buffer was 200 mM acetic acid-ammonium formate, pH 4.0.)
Column temperature: 22
Flow rate: 0.7
Injection volume: 75
Detector: F ex 470 em 550

CHROMATOGRAM
Retention time: 16.2
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: pirarubicin, doxorubicin

KEY WORDS
plasma; whole blood; daunorubicin is IS

REFERENCE
Nagasawa,K.; Yokoyama,T.; Ohnishi,N.; Iwakawa,S.; Okumura,K.; Kosaka,Y.; Sano,K.; Murakami,R.; Nakamura,H. Pharmacokinetics of pirarubicin in pediatric patients, *J.Pharmacobiodyn.*, **1991**, *14*, 222–230.

SAMPLE
Matrix: blood
Sample preparation: Condition a 6 mL Bondelut C18 SPE cartridge with 3 mL MeOH and 3 ml MeOH:phosphate buffer 1:2. 1 mL Plasma + 1 mL 10 mM pH 8 phosphate buffer containing 600 nM tetrabutylammonium bromide + 1 mL MeOH, add to the SPE cartridge, wash with 4 mL MeOH:water 25:75, elute with 3 mL 30 mM phosphoric acid in MeOH. Add the eluate to 100 μL 100 mM KH_2PO_4, evaporate to 100-400 μL under vacuum at 25°, inject a 10-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-CN
Mobile phase: Gradient. A was MeCN:10 mM KH_2PO_4 22:78. B was MeCN:10 mM KH_2PO_4 containing 6 mM phosphoric acid 70:30. A:B from 90:10 to 80:20 over 9 min.
Injection volume: 10-100
Detector: F ex 470 em 580

CHROMATOGRAM
Retention time: 10.1
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: idarubicin

KEY WORDS
plasma; SPE; daunorubicin is IS

REFERENCE
Camaggi,C.M.; Carisi,P.; Strocchi,E.; Pannuti,F. High-performance liquid chromatographic analysis of idarubicin and fluorescent metabolites in biological fluids, *Cancer Chemother.Pharmacol.*, **1992**, *30*, 303–306.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 250 μL mobile phase, extract with 3 mL MeCN for 10 min, add 100 mg NaCl, shake for 5 min, centrifuge at 995 g for 15 min, let stand at -20° for 1 h. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 10 μm Spherisorb phenyl
Column: 250 × 4.6 5 μm Spherisorb phenyl
Mobile phase: MeCN:30 mM citrate buffer adjusted to pH 4 with formic acid 30:70
Column temperature: 50
Flow rate: 1.5
Injection volume: 100
Detector: F ex 480 em 590

CHROMATOGRAM
Retention time: 8.5
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: pirarubicin, doxorubicin, doxorubicinol

KEY WORDS
plasma; daunorubicin is IS

REFERENCE
Jacquet,J.M.; Galtier,M.; Bressolle,F.; Jourdan,J. A sensitive and reproducible HPLC assay for doxorubicin and pirarubicin, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 343–348.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum

at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 234

CHROMATOGRAM

Retention time: 8.90
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood, bile, feces, tissue, urine
Sample preparation: Homogenize tissue in 4% BSA in water to a final concentration of 0.05-2 g/mL. Homogenize feces in 4% BSA in water to a final concentration of 0.03-1 g/mL. Dilute feces homogenate 20-fold, urine 100-fold, and bile 20-fold with blank human plasma. 200 μL Sample + 200 μL 6% (w/v) pH 9.5 borate buffer + 100 μL pH 2.05 water, vortex. Mix with 1 mL chloroform:1-propanol 20:80 for 5 min. (Caution! Chloroform is a carcinogen!) Centrifuge at 3000 g at 4° for 10 min. Remove the organic layer and evaporate it under reduced pressure at 43°. Reconstitute the residue in 100 μL MeCN:THF 40:1, vortex for 20 s, sonicate for 5 min. Add 300 μL water acidified to pH 2.05, vortex, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2 pellicular RP material
Column: 100 × 3 7μm Lichrosorb RP-8
Mobile phase: MeCN:THF:water adjusted to pH 2.05 with perchloric acid 30:1:80
Flow rate: 0.4
Injection volume: 50
Detector: F ex 460 em 550

CHROMATOGRAM
Retention time: 12
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: doxorubicin

KEY WORDS
mouse; plasma; brain; muscle; colon; cecum; small intestine; stomach; liver; gall bladder; kidney; lung; spleen; heart; ovary; uterus; breast; testis; epididymis; eye; daunorubicin is IS

REFERENCE
van Asperen,J.; van Tellingen,O.; Beijnen,J.H. Determination of doxorubicin and metabolites in murine specimens by high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *712*, 129–143.

SAMPLE
Matrix: blood, cells
Sample preparation: Thaw cell samples, sonicate (Branson B-12) at 50 W for 20 s. 400 μL Cell sample or plasma + 200 μL 100 mM pH 9.3 borate buffer, add 1.8 mL chloroform:MeOH 80:20, extract, inject a 200-500 μL aliquot of the organic phase.

HPLC VARIABLES
Column: 250 × 4 Lichrosorb Si-60
Mobile phase: Chloroform:MeOH:glacial acetic acid:0.3 mM magnesium chloride 72:21:2:3
Flow rate: 1.5
Injection volume: 200-500
Detector: F ex 480 em 560

CHROMATOGRAM
Retention time: 3.3
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: doxorubicin, epirubicin

KEY WORDS
plasma; normal phase; daunorubicin is IS

REFERENCE
Tidefelt,U.; Sundman-Engberg,B.; Paul,C. Comparison of the intracellular pharmacokinetics of doxorubicin and 4'-epi-doxorubicin in patients with acute leukemia, *Cancer Chemother.Pharmacol.*, **1989**, *24*, 225–229.

SAMPLE
Matrix: blood, tissue
Sample preparation: Serum. Serum + 8 mL chloroform:isopropanol 50:50, extract, centrifuge at 3000 rpm. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 5-20 μL aliquot. Tissue. Homogenize tissue in water, add 30 μL silver nitrate (33%), extract with 8 mL isopropanol. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 300 mm long 10 μm μBondapak C18
Mobile phase: MeCN:water:100 mM phosphoric acid 37:37:26
Flow rate: 1.5
Injection volume: 5-20
Detector: F ex 475 em 580

CHROMATOGRAM
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: doxorubicin

KEY WORDS
serum; rat; daunorubicin is IS; heart; liver; kidney; adrenal; brain; intestine; mouse

REFERENCE
Colombo,T.; Zucchetti,M.; D'Incalci,M. Cyclosporin A markedly changes the distribution of doxorubicin in mice and rats, *J.Pharmacol.Exp.Ther.*, **1994**, *269*, 22–27.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 150 μL Plasma + 150 μL MeCN, vortex for 10 s. Centrifuge at 3000 rpm for 5 min. Remove 200 μL of the organic layer, evaporate under reduced pressure, reconstitute the residue in 100 μL 100 mM pH 3 monobasic phosphate buffer. Inject a 10 μL aliquot. Urine. Directly inject a 10 μL aliquot. (Silanize glassware with 3% dichlorodimethylsilane in toluene, rinse with MeOH before use.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSK gel ODS/TM silica (Tosoh Co., Japan)
Mobile phase: MeCN:buffer 35:65 (Buffer was 100 mM monobasic phosphate (sic) containing 0.3% heptafluorobutyric acid, adjusted to pH 3 with NaOH. At the end of the analysis, wash the column with MeOH and MeOH:water 50:50.)
Flow rate: 1
Injection volume: 10
Detector: F ex 460 em 555

CHROMATOGRAM
Retention time: 9.8
Limit of detection: 28 nM
Limit of quantitation: 2.5 μM

OTHER SUBSTANCES
Extracted: doxorubicin

KEY WORDS
plasma

REFERENCE
Emara,S.; Morita,I.; Tamura,K.; Razee,S.; Masujima,T.; Mohamed,H.A.; El Gizawy,S.M.; El Rabbat,N.A. Utility of ion-pair chromatography for analysis of some anthracyclines in plasma and urine, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 681–692.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a C18 Sep-Pak SPE cartridge with 3 mL MeOH, 3 mL MeOH:water 50:50, and 10 mL 50 mM pH 8.9 Na_2HPO_4. 1 mL Plasma or urine + 2 mL 0.9% NaCl, mix, add to the SPE cartridge, wash with 3 mL 50 mM pH 8.9 Na_2HPO_4, elute with four 500 μL aliquots of chloroform:MeOH 2:1. Evaporate the eluates to dryness under vacuum while centrifuging, dissolve the residue in 200 μL MeCN:7 mM pH 2.6 Na_2HPO_4 40:60, vortex gently, centrifuge at 3000 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 μBondapak phenyl Radial-Pak
Mobile phase: MeCN:buffer 30:70 (Buffer was 7 mM Na_2HPO_4 adjusted to pH 2.6 with formic acid.)
Flow rate: 3
Detector: F ex 480 em 550

CHROMATOGRAM
Internal standard: daunorubicin

OTHER SUBSTANCES
Extracted: epirubicin

KEY WORDS
plasma; SPE; daunorubicin is IS

REFERENCE
Tjuljandin,S.A.; Doig,R.G.; Sobol,M.M.; Watson,D.M.; Sheridan,W.P.; Morstyn,G.; Mihaly,G.; Green,M.D. Pharmacokinetics and toxicity of two schedules of high dose epirubicin, *Cancer Res.*, **1990**, *50*, 5095–5101.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + daunorubicinone + 5 mL chloroform:isopropanol 75:25, shake mechanically for 30 min, centrifuge at 4° at 1200 g for 15 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen at room temperature. Add the aqueous phase to 2 mL 50 (plasma) or 500 (urine) mM pH 8.4 borate buffer, add 5 mL chloroform:isopropanol 75:25, shake mechanically for 30 min, centrifuge at 4° at 1200 g for 15 min. Remove the lower organic layer and add it to the residue from the first extraction, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 600 μL MeOH:500 mM phosphoric acid 50:50, vortex for 30 s,

add 2 mL N-hexane, vortex for 30 s, centrifuge, inject an aliquot of the aqueous phase (omit the hexane wash for urine samples).

HPLC VARIABLES
Guard column: 30-38 μm pellicular ODS (Whatman)
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:MeOH:10 mM pH 1.4 phosphate buffer 25:10:65
Flow rate: 0.58
Detector: F ex 480 em 560

CHROMATOGRAM
Retention time: 25
Internal standard: daunorubicin (25), daunorubicinone (35)

OTHER SUBSTANCES
Extracted: doxorubicin

KEY WORDS
plasma; daunorubicin is IS

REFERENCE
Fraier,D.; Frigerio,E.; Pianezzola,E.; Strolin Benedetti,M.; Cassidy,J.; Vasey,P. A sensitive procedure for the quantitation of free and N-(2-hydroxypropyl)methacrylamide polymer-bound doxorubicin (PK1) and some of its metabolites, 13-dihydrodoxorubicin, 13-dihydrooxorubicinone and doxorubicinone, in human plasma and urine by reversed-phase HPLC with fluorimetric detection, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 625–633.

SAMPLE
Matrix: cells
Sample preparation: Add 20 μL 33% silver nitrate solution to a suspension of 2 × 10^6 cells, agitate for 10 s, sonicate for 20 min (Bransonic 52, Vel, Belgium), add 140 μL MeCN, vortex for 5 min, cool at 4° for 30 min, centrifuge at 10000 g for 30 s, add 200 μL 200 mM pH 3 phosphate buffer, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Hibar LiChrocart RP 18 (Merck)
Mobile phase: MeCN:buffer 35:65 (Buffer was 200 mM KH_2PO_4 containing 0.2% triethylamine, adjusted to pH 3.0 with 200 mM orthophosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 237

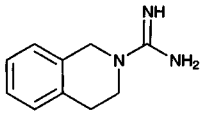
CHROMATOGRAM
Retention time: 4.0
Internal standard: daunorubicin
Limit of detection: 2 pmol
Limit of quantitation: 7 pmol

OTHER SUBSTANCES
Extracted: altretamine, doxorubicin, verapamil, S 9788

KEY WORDS
human; cells; epidermoid carcinoma; daunorubicin is IS

REFERENCE
Tassin,J.P.; Dubois,J.; Atassi,G.; Hanocq,M. Simultaneous determination of cytotoxic (adriamycin, vincristine) and modulator of resistance (verapamil, S 9788) drugs in human cells by high-performance liquid chromatography and ultraviolet detection, *J.Chromatogr.B*, **1997**, *691*, 449–456.

Debrisoquin



Molecular formula: $C_{10}H_{13}N_3$
Molecular weight: 175.23
CAS Registry No.: 1131-64-2, 581-88-4 (sulfate)
Merck Index: 2901
Lednicer No.: 2 374

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 262

CHROMATOGRAM

Retention time: 4.07
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, saliva, urine

Sample preparation: Plasma. 1 mL Plasma + IS + 1 mL MeOH + 1 mL 2 M HCl, mix, centrifuge. Remove the supernatant and add it to 1 mL 2 M HCl, cool, wash with 8 mL diethyl ether. Add the aqueous layer to 1 mL 5 M NaOH. Remove a 1 mL aliquot and add it to 500 μL saturated sodium bicarbonate solution, add 500 μL MeOH, add 500 μL acetylacetone, heat at 96° for 2.5 h, cool to room temperature, add 3 mL 5 M NaOH, extract with 8 mL diethyl ether. Remove the organic layer and add it to 500 μL 2 M HCl, shake mechanically for 15 min, centrifuge. Remove the aqueous layer and add it to 500 μL 5 M NaOH, cool, extract with 8 mL diethyl ether. Remove the organic layer and evaporate it to dryness at 45°, reconstitute the residue in 20 μL MeOH, inject an aliquot. Saliva, urine. 1 mL Saliva or urine + 2 (saliva) or 10 (urine) μg IS in MeOH + 500 μL saturated sodium bicarbonate solution + 500 μL MeOH + 500 μL acetylacetone, heat at 96° for 2.5 h, cool to room temperature, add 3 mL 5 M NaOH, extract with 8 mL diethyl ether. Remove the organic layer and add it to 500 μL 2 M HCl, shake mechanically for 15 min, centrifuge. Remove the aqueous layer and add it to 500 μL 5 M NaOH, cool, extract with 8 mL diethyl ether. Remove the organic layer and evaporate it to dryness at 45°, reconstitute the residue in 20 μL MeOH, inject an aliquot.

HPLC VARIABLES

Guard column: C8 (Merck)

Column: 100 × 8 μBondapak C18 in a Z-module

Mobile phase: MeOH:water containing 10 mM sodium 1-pentanesulfonate, adjusted to pH 3.5 with orthophosphoric acid

Flow rate: 1.5

Injection volume: 25-200

Detector: UV 248

CHROMATOGRAM

Retention time: 7.4

Internal standard: guanoxan hemisulfate (Pfizer) (5.6)

Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

derivatization; silanize all glassware with hexamethyldisilazane; plasma; comparison with GC; pharmacokinetics

REFERENCE

Chan,K. Comparison of gas chromatographic and high-performance liquid chromatographic assays for the determination of debrisoquine and its 4-hydroxy metabolite in human fluids, *J.Chromatogr.*, **1988**, *425*, 311–321.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide,

sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: Condition a C18 SPE cartridge with 4 mL MeOH and 4 mL water. Filter (0.45 μm) urine, adjust pH of filtrate to 5 with 100 mM HCl, add a 1 mL aliquot to the SPE cartridge, wash with 3 mL water, wash with 1 mL MeOH:water 10:90, elute with 1 mL MeOH:water 90:10, inject an aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 Spherisorb C8

Column: 250 × 4.6 5 μm Spherisorb C8

Mobile phase: MeCN:buffer 70:30 (Buffer was 8 mM KH_2PO_4 adjusted to pH 5 with 2 M KOH.)

Flow rate: 1.5

Detector: UV 208

CHROMATOGRAM

Retention time: 7.5

Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, 4-hydroxydebrisoquine

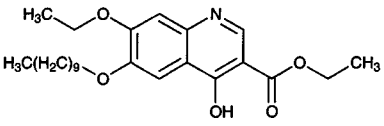
KEY WORDS

comparison with capillary electrophoresis; SPE

REFERENCE

Cifuentes,A.; Valencia,J.; Sanz,E.; Sánchez,M.J.; Rodríguez-Delgado,M.A. Separation and quantitation of debrisoquine and 4-hydroxydebrisoquine in human urine by capillary electrophoresis and high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 389–396.

Decoquinate



Molecular formula: $C_{24}H_{35}NO_5$
Molecular weight: 417.55
CAS Registry No.: 18507-89-6
Merck Index: 2910
Lednicer No.: 2 368

SAMPLE

Matrix: feed
Sample preparation: 10 g Feed + 50 mL calcium chloride in MeOH, shake vigorously for 20 min, centrifuge at 1000 rpm for 2 min, remove the supernatant and extract the residue twice with 50 mL portions of calcium chloride in MeOH. Combine all the supernatants and add them to 100 mL 500 mM HCl, extract with three 20 mL aliquots of chloroform. Combine the chloroform extracts and wash them with 20 mL water. Extract the aqueous layer three times with 15 mL portions of chloroform. Combine all the chloroform layers and evaporate them to dryness under vacuum at 40°. Reconstitute the residue in MeOH: chloroform 50:50, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.5 3 μm Spherisorb ODS
Mobile phase: Gradient. A was MeOH:water 95:5 containing 0.001% acetic acid and 1% magnesium sulfate heptahydrate. B was MeOH. A:B 5:95 to 80:20 over 10 min (convex gradient, code 0.3, Perkin-Elmer) then to 95:5 over 5 min (linear), then to 5:95 over 5 min (linear), re-equilibrate for 10 min.
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: F ex 314 em 390

CHROMATOGRAM

Retention time: 9
Limit of quantitation: <1000 ng/mL

OTHER SUBSTANCES

Simultaneous: nequinate, buquinolate
Noninterfering: amprolium, arprinocid, clopidol, dimetridazole, sulfaquinoxaline

REFERENCE

Hobson-Frohock,A. Determination of decoquinate in poultry feed, *Analyst*, **1982**, *107*, 1195–1199.